

The University of Chicago

A STUDY OF
THE MAXIMUM CAPACITY OF THE
VASCULAR SYSTEM

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
DECEMBER, 1941

By
EDWIN LEE SMITH

CHICAGO, ILLINOIS

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INTRODUCTION

It was considered of interest to determine the maximum capacity of the vascular system of an animal which is compatible with life. As an approach to the problem, large amounts of whole dog blood were injected into dogs. Data on the amounts tolerated and on effects of these massive transfusions are presented in this paper.

The literature did not seem to contain any data which satisfactorily define the upper limit of volume which can be given in a transfusion without death of the animal. A certain amount of experimental data is available on the results of rather large transfusions. Krumbaahr and Chanutin (1), using dogs and rabbits, and Robertson (2), using rabbits, injected total amounts of blood larger than any used in the experiments reported herein. However, the injections were made in small doses over periods of many days. Therefore, it seems that any inferences as to maximum blood volume are unjustified. Boycott and Oakley (3) and Campbell (4) have reported faster transfusion rates, but the volumes they injected were smaller, and did not produce death in their animals.

METHOD

Acute Experiments

For each experiment one small dog (4-8 kilograms) was used as recipient and one or more large dogs as donors of blood. The dogs were anaesthetized with 300 mgms/kg/sodium barbital (intravenously or intraperitoneally).

A tracheal cannula was inserted in both dogs. In the recipient, the carotid artery was cannulated, and blood pressure was recorded continuously on a moving kymograph by means of a membrane manometer. A solution containing 8% of sodium citrate in distilled water was used as anticoagulant in the manometer. Respiration was also recorded from a chest pneumograph connected to a tambour.

The transfusion of whole blood from donor to recipient was performed as follows: Either the saphenous vein or the femoral vein of the recipient was exposed. (The femoral vein was used in the first experiments, but the saphenous vein was found to be more convenient.) A 16 gauge needle was inserted, pointing centrally, into the vein and held in position with a bulldog clamp.

The carotid artery of the donor dog was isolated and tied peripherally. Blood was withdrawn from the arterial stump through a 16 gauge needle into a 50 cc syringe.

In order to prevent clotting of the blood during the short time required for the transfer, the inside of the clean syringe was moistened with 4% sodium citrate. In general, two minutes or less elapsed between the beginning of withdrawal of each 500 cc portion of blood to the end of its injection. The time intervals

between injections varied in the different experiments.

Chronic Experiments

These dogs, donors as well as recipients, were anaesthetized with 35 mgms/kg/sodium pentobarbital. Customary precautions were taken to prevent infection during the transfusion. The blood was handled in the same manner as described for the acute experiments. It was taken from the carotid artery of the donor and injected into the saphenous vein of the recipient.

The normal blood volume and hematocrit of each chronic recipient dog was determined before the transfusion. After transfusion, blood volume and hematocrit changes were followed.

Blood Volume Method

All blood volume determinations were made by the method of Gregerson, Gibson and Stead (5), as modified by Gibson and Evans (6). Having previously trained the chronic dogs, it was then possible to make blood volume determinations without the use of anaesthesia, analgesia, or restraint.

TABLE 1

ACUTE EXPERIMENTS ON SURVIVAL TIME AFTER MASSIVE
TRANSFUSIONS OF BLOOD

Exp.	Weight in kgm	Volume of blood injected cc/kilo	Rate of injection cc/kg/min	Survival after last injection
T	7.73	84.1		6 wks.
D	5.9	84.7	3.38	3 wks
5B	6.36	98	3.92	1 hr. sacrific'd
4	6.81	102	1.13	2½ hr. sacrific'd
B	6.36	102	8.51	1 wk. Died
7	6.59	106	1.41	7 hrs. sacrific'd
J	6.59	106	3.9	3½ hrs. Died
13	6.36	157	5.23	10 min. Died
12	4.1	158	5.2	5 hrs. sacrific'd
6	5.0	170	4.85	0
11	5.45	172	3.12	0
9	5.68	176	1.76	2½ hrs. sacrific'd
8	5.68	176	5.01	1 hr. sacrific'd
10	4.54	187	1.24	2½ hrs. sacrific'd
5	4.7	230	4.18	40 min. Died

RESULTS

Normal Dogs

In Table 1 the results of massive injection of whole dog blood in fifteen dogs are presented. The dogs of this series are designated throughout the paper as normal dogs in contradistinction to the dogs of the second series, from which certain organs were removed. It should be pointed out that dogs T, B, D, and J represent chronic experiments, which will be discussed more fully later, but which are included there because they are comparable to the acute normal animals. The amounts and rates of injection were varied. In some cases (listed as O), in the column headed survival time, the injection was continued until the animal succumbed. In other cases, the injection was stopped in order to see if the animal could survive the given amount for at least an hour.

The smallest transfusion to produce death within a few minutes was 157 cc/kg (exp. 13). The largest transfusion that any dog survived for one hour or more was 187 cc/kg. In the seven dogs receiving amounts between these two limits, which might be called the critical range, there seems to be no definite relation between the survivals and the volume of the transfusion.

Since the rates of injection varied in these dogs, one might expect to find some correlation between the rate of injection and mortality within this comparatively narrow range of volumes injected. There appears to be such a correlation among the dogs that died. However, among the dogs that lived, one animal (exp. 8) survived one of the largest transfusions at one of the fastest rates.

Table 2 gives a summary of the outstanding features of the blood pressure recordings of the eleven normal dogs from the acute experiments. There seems to be a direct correlation between the amount injected and maximum blood pressure rise. Four dogs (#4, #7, #11, #10) do not follow this generalization. Of these, the following may be said:

(a) Dogs #4, #10, and #7 received blood at very slow rates. The maximum rise in blood pressure in these three dogs came early in the transfusion. In the other eight dogs, the maximum rise was after the last injection.

(b) Dog #4 was given blood which had been concentrated by removal of over half of the plasma.

(c) Dog #11 reacted to the first 500 cc injection of blood with a severe drop in blood pressure, which may have been due to incompatibility. The dog's blood pressure rose progressively after this initial drop and attained a normal level shortly before death of the animal. This was the only dog in which any depressor reaction was noticed.

The height of the blood pressure rise in this series of animals does not bear any close relation to the incidence of death. The dogs with the highest rise above normal and the dogs with the highest absolute blood pressure were not any more susceptible than the others.

One of the constant features of all the experiments was the early appearance of an extensive erythema. At some time during the transfusion defecation almost invariably occurred. It was, however, rather surprising, even in experiments lasting several hours, to note the complete absence of urination in most of the dogs. It was not an infrequent occurrence that, shortly before death, a small quantity of bloody fluid came out of the tra-

cheal cannula.

TABLE 2
MAXIMUM BLOOD PRESSURE RISE FOLLOWING MASSIVE INJECTION

Exp.	Volume of blood injected cc/kilo	Rate of injection cc/kg/min	Maximum mean blood pres- sure rise
5B	98	3.92	30
4	102	1.13	50
7	106	1.41	15
13	157	5.23	50
12	158	5.2	60
6	170	4.85	60
11	172	3.12	0
9	176	1.76	70
8	176	5.01	70
10	187	1.24	20
5	230	4.18	70

On post mortem examination of the heart, the right auricle showed varying degrees of distension. The left auricle and the ventricles were approximately normal in size. The lungs were usually congested and contained watery fluid. The liver was markedly distended in all cases. The vessels of the gut were engorged with blood, but hemorrhagic areas were rarely encountered. There was more than the normal amount of fluid in the abdominal cavity, and it was sometimes tinged with red.

Chronic Dogs

On the basis of the data from the acute experiments, three dogs were injected with amounts of blood which they might be expected to survive. These dogs were then cared for and

studied. The protocols of the chronic experiments are as follows:

Dog T: Dog weighed 7.7 kg. On 6/28/41, 650 cc (84.1 cc/kg) of whole blood were injected. The dog survived the transfusion. A slight edema occurred but disappeared in 12 hours. No ill effects were observed. On 8/2/41, 35 days after injection, the dog was alive and healthy, with a hematocrit of 45.

Dog D: Dog weighed 5.9 kg. On 7/19/41, 500 cc (84.7 cc/kg) of blood were injected over a period of 25 minutes. (3.38 cc/kg/min.) Plasma volumes and hematocrits were determined both before and after transfusion.

Dog D: 5.9 kg

Days after injection	Hematocrit	Plasma Vol.	Blood Vol.
Control	42.1	340.8	587
0	68.4	448 ?	1417 ?
2	64.9	300	854
4	65.2	306	879
6	61.0	312	800
8	60.9	280	718

Dog B: Dog weighed 6.36 kg. On 7/12/41, 650 cc (102.2 cc/kg) of blood were injected over a period of 12 minutes (8.51 cc/kg/min.). 7½ hours after the last injection the dog appeared to have recovered from anaesthetic and operation. The swelling around the eyelids, and other earlier evidences of edema, had disappeared.

The dog was lively and ate well until the 5th day. She then became listless and refused to eat. When offered food she vomited mucous and bile. Two days later the dog died. The cause of death was undetermined. A post mortem examination showed no gross abnormalities of the lung, heart, liver, or kidney. The lungs weighed 70 gms., spleen weighed 55 gms., and liver weighed

360 gms.

Changes in plasma volumes and hematocrits were followed until the death of the animal.

Dog B: 6.36 kg

Days after injection	Hematocrit	Plasma Vol.	Blood Vol.
Control	56.4	368	686
0	64.2		
7½ hrs.	69.1		
3	68.9	265	856
5	70	222	742
7	DIED		

Mechanisms

The following experiments were performed in an attempt to determine the nature of the adjustment to the increased blood volume that was being made by the dog.

Splenectomy was done on four dogs immediately before transfusion was started. These four experiments are presented in Table 3. There were no experiments on normal dogs with transfusion volumes closely similar to dogs #15 a and #15b. However, the transfusions administered to these two dogs fall on the lower border of the "critical range" (150-200 cc/kg). One of these dogs lived, and one died. These findings are not significantly at variance with the normal.

The large volume and rate tolerated by the other two splenectomized animals suggest even more strongly that splenectomy did not reduce the ability of the dog to adjust to the added blood.

A second type of experiment was designed to test the role of reflex adjustments to the increased blood volume. Three such experiments were performed (see Table 4). In all three experiments, double vagotomy was performed preliminary to transfusion.

In the first of these (#14b), an attempt was made to denervate the carotid sinuses by stripping the appropriate region of arteries, then painting with 85% phenol. In the second one of the series (#17), a similar procedure was carried out but, upon testing, the carotid sinus reflex seemed to be still active, so the sinuses were isolated from the circulation by tying. The third experiment differed from the other two in that the carotid sinuses were completely extirpated.

The fact that one of the dogs required 198 cc/kg to be killed makes it impossible to state, from these experiments, that these mechanisms are invariably necessary in coping with increased blood volume. It is possible that other pressor-receptor areas were acting, just as it is possible that the dog which died with

TABLE 3
MASSIVE INJECTION INTO SPLENECTOMIZED DOGS

Exp.	Wt. in kilos	cc/kg	Rate	Maximum blood pressure rise	Survival Time
15A	5.2	144	4.8	65	0
15B	7.7	149	.3	70	5 hrs. sac'd
14A	4.1	207	2.34	105	0
16	5.2	234	10.4	65	20 min.

TABLE 4
MASSIVE INJECTIONS INTO DOGS WITH CAROTID SINUSES
DENERVATED AND VAGI CUT

Exp.	Wt. in kilos	cc/kg	Rate	Maximum blood pressure rise	Survival Time
14B	6	100	10	60	0
18	5.9	161	5.03	105	0
17	7.8	198	2.34	60	0

100/kg of blood depended more than the others on its carotid sinus-aortic arch reflexes.

It is interesting to note that, in the dogs whose carotid sinuses were denervated and vagi nerves cut, the maximum rise in blood pressure came before 200 cc of blood had been injected, while in the dogs that had been splenectomized, the maximum rise in three of the four came at the end of injection (and the fourth only after 400 cc). The average blood pressure rise in the normal dogs is less than in those whose carotid sinus reflexes had been abolished. Also, at the death of all three of the dogs whose carotid sinuses were stopped, the circulation failed before the respiration, whereas, two of the three splenectomized dogs died of respiratory failure.

Organ Weights

A comparison of the weights of the spleens, liver, and lungs of the injected dogs with the weights of the organs of normal, uninjected dogs, showed that the former were definitely heavier. In absolute terms the livers increased most in weight.

DISCUSSION

Among the most important variable factors which determine the ability of a given animal to withstand massive transfusions are amount and rate of injection. These two factors were varied over a considerable range in the work reported here. The data show that survival is, to a high degree, correlated with them, but that the correlation is not perfect. This is not surprising in view of the fact that there is a great number of other factors which were not controlled or evaluated, such as the physical condition of the animal, the depth of anaesthesia, and the formation of emboli from the locus of injection. Furthermore, individual variations among dogs, as, for example, the very variable response to carotid sinus denervation, the possibility of various degrees of incompatibility reactions, and finally the probably very variable rate at which plasma is lost from the blood vessels. (Cf. below on this last factor.)

The ratio of the average blood volume of normal dogs to their weight has been reported differently by many workers, but probably lies somewhere between the 7.6% of Harris (7) and the 11.91% of Powers, Bowie and Howard (8). If one assumes the correct value as 10%, the "critical range" of transfusion volumes postulated from the acute experiments lies between $1\frac{1}{2}$ and 2 times that of the average blood volume; that is to say, an animal's blood volume can often be tripled before death occurs. The results of the chronic experiments, however, indicate that, if complete recovery (instead of survival for one hour) were used as a criterion, this range would have to be lowered somewhat.

As mentioned above, plasma is lost from the vessels at a rate which probably varies greatly from dog to dog. Unfortunately, a most serious handicap presented itself in determining the total blood volume immediately at the end of the transfusions. Whereas, in control determinations as well as in determinations made some time after transfusion the Evans dye method yielded very consistent results (which were, moreover, in close agreement with the values found in the literature [9]), the method failed completely when determinations were made at the end of transfusion. The values found then were far too low or far too high, at times considerably higher than the sum of the recipient's blood volume, as determined before the experiment, and that of the blood injected.

This failure of the method might be explained on the assumption, particularly in those dogs where the values obtained were too high, that some of the dye was lost through leakage from the abnormally dilated capillaries. This hypothesis might be checked by analysis of the edema fluid in the abdominal cavity and pulmonary alveoli, or else by plasma protein determinations: should the plasma protein content be found to be lower than it should be following such hemoconcentration, it would seem reasonable to assume that dye particles left the blood stream in the same manner in which the plasma proteins were lost.

A second explanation for the obvious unreliability of the plasma volume determinations might be extreme sluggishness of the circulation or of certain parts of the circulation which might prevent complete mixing within the time allowed (10 min.). On this assumption, both distortedly high and distortedly low values might be found, as was actually the case.

If it should be confirmed that the dye injection method is not applicable immediately after transfusion, the following in-

direct method can be offered as a possible solution to the problem: If one determines blood volume and hematocrit of the recipient dog before transfusion, the total volume of blood injected, and the hematocrits of the injected blood as read on each 500 cc of blood drawn, one can then calculate the total cell volume present before injection and the total cell volume injected. This, together with a hematocrit determination made immediately at the end of transfusion, permits one to calculate the total blood volume at this time.

The only assumption made here is that during the time of transfusion there has been no considerable loss of cells, e.g., through hemorrhage or through destruction. Hemorrhage, as determined by crude autopsies, was in no case very extensive, and destruction would not seem to be a significant factor during a time interval of two hours at the most. (Cf. on this point, Boycott and Oakley (10) who found that the rate of destruction rises only after a considerable delay when experimental polycythemia is produced.)

Thus, the work, as so far completed, shows the injected blood to be distributed in three places. A small amount is accounted for by the weight increase in the spleen, in the lungs, and particularly in the liver (whose increase in weight was found to be very much higher than that of the other organs). A second small portion is accounted for by the edema in lungs and abdominal cavity. The greatest portion is still in the vascular system. Vasodilatation could be directly observed in all the skin capillaries, and hyperemia seemed evident in the profuse bleeding from the cut surfaces of muscles and organs.

It might be expected that in animals whose vascular systems had been so over loaded the most probable cause of death

would be heart failure. In the animals reported in this series, however, respiration failed before the heart more often than not. The first explanation for this result that comes to mind is that of cerebral edema. With this idea in mind, it might prove of value to record intracranial pressure in experiments of this nature.

SUMMARY

1. This work was done in an attempt to determine the maximum amount of blood which the vascular system of a dog can tolerate. Of 15 normal dogs, the seven receiving 150 cc/kg or less lived for at least one hour after the last injection. Of the seven receiving 150-200 cc/kg, four lived at least an hour and three died. One dog receiving over 200 cc/kg died.

2. Two dogs receiving 85 cc/kg have lived several weeks. One dog receiving 102 cc/kg lived one week. One dog receiving 106 cc/kg lived $2\frac{1}{2}$ hours.

3. After transfusion, there was an immediate loss of plasma, resulting in a high hematocrit. The initial rise in blood volume was followed at once by a rapid fall to a level still exceeding the control volumes. This increased volume was maintained for several days, although the hematocrit fell gradually.

4. In dogs whose spleens had been removed just before transfusion, the lethal transfusion volume was approximately the same as in normal dogs.

5. Double vagotomy, plus denervation or complete removal of the carotid sinuses, seemed to make little if any difference in the amount of blood that the dogs could tolerate.

6. After a massive transfusion, more of the excess blood is to be found in the liver than is to be found in the spleen or lung.

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